proteins which undergo rotational diffusion around the membrane normal, but whose mobility is otherwise restricted by interactions with the membrane phospholipids. The spectra of membrane proteins alone and in complex with other proteins and ligands set the stage for structure determination and functional studies.

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Insight into the Thermodynamics and Equilibrium Kinetics of the Interaction between Transmembrane α-Helices in the Membrane Domain of ErbB4

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Interactions between the transmembrane (TM) α-helices in the membrane domains (MD) of integral membrane proteins are believed to determine their spatial structure and functionality. Nevertheless, the basic principles underlying such interactions still need to be elucidated. In the present work, the interaction between the TM segments in two-helical MD of the receptor tyrosine kinase ErbB4 was investigated by means of solution NMR spectroscopy in lipidic bicelles. According to the obtained data, the α-helical TM domains of the receptor associate via the double motif $A^{655}GxxGG^{660}$, forming the parallel dimer, stabilized by the polar contacts. The slow character of dimer-monomer transition of the ErbB4 TM domains permitted to access the thermodynamics and equilibrium kinetics of the dimerization. Lipidic bicelles appeared to be an ideal solvent in terms of dimer-monomer equilibrium of the ErbB4 TM domains, which allowed to measure the free energy of their dimerization equal to −1.4 kcal/mol. Noteworthy, the dimerization constant began to increase dramatically when more than one peptide on average were induced to reside in one bicelle (''saturated bicelles''). Enthalpy, entropy and heat capacity of the dimerization were obtained from the temperature dependence of the dimerization constant in ''saturated bicelles''. As was shown, the dimerization of TM domains of ErbB4 is an endothermic process, going with the substantial growth of entropy and heat capacity of the system, suggesting the important role of lipids in the TM helix-helix interactions. The temperature dependence of linewidths of NMR signals was interpreted in terms of the reaction rates and the transition state theory to derive the free energy, entropy and enthalpy of the transition state and to estimate the contribution from the lipid-protein and protein-protein interactions into the free energy of dimerization.

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Structure and Dynamics Provides Direct Insight into Proapelin Processing

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Apelin is the peptide ligand for a class A G-protein coupled receptor (GPCR), denoted here as AR. The apelin-AR system has been shown to have roles in regulation of fluid homeostasis; angiogenesis during tumour formation; adipoinsular axis function; and in regulation of the cardiovascular system and central nervous system. Apelin is expressed as a 77-residue preproprotein which is truncated by various unidentified processing pathways to bioactive forms ranging from 13-36 residues, all of which include the extreme C-terminus of the preproprotein. Such a promiscuity in length of bioactive peptide, with forms at lengths from 13 to 36 residues observed in vivo, is highly unusual and may provide insight into the ability of the apelin-AR system to exert disparate physiological effects. Until now, no specific information is available about the processing pathway(s) involved in apelin production. We have cloned and expressed human proapelin, the C-terminal 55-residue component of preproapelin produced by postulated N-terminal signal peptide removal. This was produced in E. coli as a fusion protein with an N-terminal hexahistidine tag and TEV protease cleavage site suitable for affinity purification and high yield isolation following TEV protease cleavage. Proapelin is a highly basic peptide with multiple dibasic sites, any and all of which are potential sites of cleavage for the proprotein convertase (PC) class of enzymes. We present here a comprehensive structural and dynamics characterization of proapelin using CD, fluorescence and NMR spectroscopy. These findings are allowing us to probe the accessibility of the dibasic sites in proapelin to PC enzymes. This will facilitate the identification of the most likely PC enzymes involved in proapelin processing, allowing for direct validation by biochemical assays.

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Function and Dynamics of Thrombin by NMR

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Thrombin is a serine protease that plays an essential role in the blood-clotting cascade, cleaving soluble fibrinogen to insoluble fibrin and activating platelets. Thrombin is also involved in tissue repair, angiogenesis and inflammation via the activation of the PAR-1 and PAR-3 receptors. In its positive feedback loop, thrombin activates factors XI, V, and VIII to promote further clotting. When thrombin binds thrombomodulin (TM) at anion binding exosite 1 (ABE1) the feedback loop becomes negative by activating protein C, which in turn inactivates factors VIIIa and Va, decreasing the rate of thrombin activation. The mechanism of the thrombin specificity change from procoagulant to anticoagulant remains elusive since crystal structures of thrombin bound to TM show no structural change in the active site. NMR provides the residue specific resolution needed to study the dynamics and the contribution to thrombin function. Near complete assignments of thrombin backbone resonances have been achieved using high-field TROSY based methods to study [2H,15N,13C] thrombin. Relaxation and relaxation dispersion experiments performed on thrombin covalently inhibited with PPACK show functional regions with increased dynamics, including ABE1, the γ-loop, and ABE 2. Analysis of residual dipolar couplings in light of accelerated molecular dynamics simulations indicated a structural ensemble that is highly mobile in several timescales, providing a path forward towards understanding the role of dynamics and conformational fluctuations on catalysis and substrate interactions in thrombin.

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A PagP Fusion Protein System for the Expression of Intrinsically Disordered Proteins in E. Coli

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Optimizing protein expression is a key step for NMR spectroscopy, which typically requires milligram quantities of isotope-labeled protein.
Human cardiac troponin I (cTnI) is a 210-amino acid protein that accumulates in intracellular inclusion bodies when overexpressed in E. coli. Our initial attempts at producing an isolated N-terminal fragment, cTnI[1-71], resulted in negligible expression. This was due to proteolytic degradation, as evidenced by the expression of a fusion construct, GB1-cTnI[1-71], yielding GB1 alone. In order to target cTnI[1-71] to inclusion bodies, we fused it to Comamonas testosteroni ketosteroid isomerase (KSI), a hydrophobic 125-amino acid protein, using the commercially available pET31b expression vector.
The KSI-cTnI[1-71] fusion protein was expressed in inclusion bodies and purified to a reasonable yield. However, when a tandem dimer, KSI-cTnI[1-71]-cTnI[1-71], was expressed, only a single band corresponding to the molecular weight of KSI alone was observed, suggesting that it was susceptible to intracellular proteases.
Beta barrel proteins of the Gram negative bacterial outer membrane accumulate in inclusion bodies when overexpressed without their N-terminal signal sequence. A fusion construct involving the E. coli outer membrane enzyme, PagP, (PagP-cTnI[1-71]) expressed robustly in inclusion bodies. Moreover, the tandem dimer, PagP-cTnI[1-71]-cTnI[1-71], also expressed well.
Using a beta barrel protein as a fusion partner has several theoretical advantages for expressing intrinsically disordered proteins. There is no possibility for the protein to fold outside of the membrane environment. Furthermore, beta barrel membrane proteins are not extremely hydrophobic, since the interior of the barrel is typically hydrophilic. Hydrophobic proteins tend to have limited yield, can be toxic to cells, and may interfere with later purification or processing steps. Thus fusion to PagP or other beta barrel membrane proteins represents a promising new alternative for difficult-to-express disordered proteins.

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Structural and Binding Studies of the C-Terminal Domains of Yeast TFIIF Subunits Tfg1 and Tfg2

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The general transcription factor TFIIF plays essential roles at several steps during eukaryotic transcription. While several studies have offered insights into the structure/function relationship in human TFIIF, much less is known about the yeast system. Here, we describe the first NMR structural and binding studies of the C-terminal domains (CTDs) of Tfg1 and Tfg2 subunits of Saccharomyces cerevisiae TFIIF. We used the program CS-ROSETTA to determine the three-dimensional folds of these domains in solution, and performed binding studies with DNA and protein targets. CS-ROSETTA models indicate that the Tfg1 and Tfg2 C-terminal domains have winged-helix architectures, similar to the human homologs. We showed that both Tfg1 and Tfg2 CTDs interact with double-stranded DNA oligonucleotides, and mapped the DNA binding interfaces using solution NMR. Tfg1-CTD, but not Tfg2-CTD, also binds to yeast FCP1, an RNA polymerase II-specific phosphatase, and we delineated the interaction surface with the CTD of FCP1. Our results provide insights into the structural basis of yeast TFIIF function and the differential roles of Tfg1 and Tfg2 subunits during transcription.